

METHODS FOR USING PET MEASURED METABOLISM TO DETERMINE COGNITIVE IMPAIRMENT

The present application relates to the use of Positron Emission Tomography (PET) imaging to produce images of brain metabolism useful in diagnosis and treatment of mental impairment from brain pathology resulting from injury, disease, or other cause. The image intensity values derived from PET scans of selected regions of the brain are combined using various mathematical and analytic techniques to identify mild cognitive impairment and construct a cognitive decline index that is used to diagnose and monitor the progress of treatment of cognitive impairment.

PET is an imaging technology that allows physicians and researchers to observe and analyze the chemical functioning of an organ or tissue, rather than anatomical structure as in MRI and CT. By examining cellular and metabolic activity, this imaging tool is vital to diagnosing and assessing the progression of diseases such as cancer, 5 Parkinson's disease, Alzheimer's disease, heart disease, stroke and numerous other common afflictions. Furthermore, PET allows for continuous and immediate monitoring of the effectiveness of medications and drugs.

There are a number of brain disorders that may be analyzed using functional PET imaging, including degenerative brain disorders such as Alzheimer's Disease (AD), 10 Jacob-Kreutzfeldt disease and cerebral dysfunction caused by stroke, drug abuse and closed head injury. These diseases and conditions all show diminution of cognitive ability, loss of memory and may also show personality disorder. Measurement of cognitive decline or dysfunction is a powerful tool that can be used to identify, monitor and identify changes in these conditions. This cognitive decline or dysfunction is 15 referred to herein as mild cognitive impairment (MCI).

Alzheimer's disease (AD) is representative of a number of diseases result from chronic, pervasive processes that begin well before memory loss and concomitant cognitive impairment is noticed by the patient. In addition to AD, these diseases include Parkinson's disease, Huntington's disease, Pick's Dementia, Jakob Kreutzfeldt 20 syndrome and Dementia with Lewy bodies. MCI resulting from head injury, patient intake of drugs or intake of alcohol exemplify a few of the conditions that will benefit from diagnostic methods that indicate the earliest possible detection.

Current methodologies for early detection and diagnosis of MCI and the degenerative diseases that induce MCI early in their development take several approaches, including genetic analysis, neuropsychological tests, and functional neuroimaging. Measurement of brain metabolism *in vivo* has been shown to be a very sensitive method to detect even early cognitive changes. In fact, several previous reports indicate that it is possible to detect brain functional changes across groups of patients before subjective symptoms or neuropsychological impairment occurs (Small GW, et al, (2000). PNAS 97(11):6037-6042, Reiman EM, et al, (2001). PNAS 98(6):3334-3339, De Leon MJ, et al, (2001). PNAS 98(19):10966-10971). However, reliable methods of detecting MCI in individual patients at a clinical level are lacking. Detection of this degenerative process in pre-morbid states would enable early treatment with medication to enhance and prolong quality of life, provide an answer to patients' questions regarding their potential for cognitive decline, help them plan and prepare for the future, and hopefully one day to prevent the disease altogether.

The combination of imaging capability based on metabolic activity and computing techniques with capacity to store large amounts of information and perform pattern recognition provides the potential for identifying parts of the brain malfunctioning in disease or injury. The instant invention utilizes this combination to construct indices of MCI by incorporating statistical tools to analyze the departures from normality of the image intensity patterns of diseased brains. In one embodiment, neural network techniques are employed to enhance the analysis of intensity patterns accompanying disease or injury.

Neural networks have great applicability in capturing associations or discovering regularities within a set of patterns, where the volume, number of variables or diversity of the data is great; the relationships between variables are incompletely understood; and the relationships are difficult to describe adequately with conventional approaches. Neural Networks comprise a collection of computational techniques that are universally applicable approximators that work well in modeling systems that have high variability and a concomitant tolerance to error.

The individual parts are called neurons. They mimic as does the network they comprise the action of individual neurons and their network in the human brain. A neural network is an interconnected group of neurons. The prime examples are

biological neural networks especially in the human brain. In modern usage, the term most often refers to the artificial neural networks described herein as computational

Artificial neural networks (ANNs) are not sequential or necessarily deterministic. There are no complex central processors, rather there are many simple
5 processors that generally do nothing more than take the weighted sum of their inputs from other processors, perform a simple, specified function and pass the result on to other single purpose processors. ANNs do not execute programmed instructions; rather, they respond in parallel (either simulated or actual) to pattern of inputs. There are also
10 no separate memory addresses for storing data. Instead, information is contained in the overall activation "state" of the network. "Knowledge" is represented by the network itself, which is more than the sum of its component parts.

United States Patent 5,262,945, DeCarli, et. al., presents some of the earliest work on technology generally known as automated image segmentation. The DeCarli patent addresses the problem inherent in the technology that measuring volume of a
15 structure in the brain had to be done manually, for example, by drawing two lines across a ventricle in a given brain image slice, in a cross formation, and then calculating volume based on those distances. It was possible and time consuming to outline the entire volume in that slice. Accuracy improved with more slices taken, however, the distinct boundary between ventricle and brain tissue had to be drawn by hand.
20 Utilization of the properties of the digital images delivered by MRI enabled differentiation between tissues based on the difference of image intensities. Image quality and contrast led to the development of methods to select one pixel in the image (a seed-point) which was then used to define a region based on contiguous pixels of a similar intensity. This in turn led to the ability to implement software to automatically
25 place seed-points at random, and hence fully automate segmentation of the image into various tissues: scalp, CSF, gray matter, and white matter.

The MRI based DeCarli patent utilizes measurements of volume to define regions of interest (ROI) based on histogram intensity of threshold-defined structures. The DeCarli patent teaches determination and identification of disease presence by
30 searching for differences in volume and teaches monitoring of disease progression by observing volumetric changes. The DeCarli patent can be used therefore to determine volumes of various regions in the brain and to detect AD and other disorders that may lead to volumetric changes occurring at later stages of the disease.

This DeCarli method may be distinguished from the instant PET based patent which uses data from the measurements of glucose metabolism to define three dimensional spherical volumes of interest (VOI) of 1 cm diameter where the center of the sphere is located by using a mathematical treatment based on statistical parametric mapping (SPM). The 1 cm diameter, spherical VOI is roughly equivalent to a cube of 125 volume elements (voxels); i.e. a cube 5 voxels on a side where each voxel is a 2 mm isotropic volume element.

Statistical parametric maps are spatially extended statistical processes that are used to test hypotheses about regionally specific effects in neuroimaging data. The most established sorts of statistical parametric maps are based on linear models, for example analysis of covariance (ANCOVA), correlation coefficients and t-tests. Application of SPM brings together two well established bodies of theory (the general linear model and the theory of Gaussian Fields) to provide a complete and simple framework for the analysis of imaging data. SPM is a software package that consists of a collection of tools used to process and analyze 3D functional brain image data. SPM runs in MATLAB® (Mathworks, Inc., Natick, MA) shell (<http://www.mathworks.com/products/matlab/>). SPM is used to spatially normalize and spatially filter the brain image data (processing) and then to compare two groups of subjects and statistically analyze the results. These results are subsequently used to provide loci for sampling with MARSBAR, a “plug-in” accessory program for SPM (<http://marsbar.sourceforge.net/>), which actually does the intensity sampling of the 5 mm radius spherical volumes of interest. The 3D matrix calculations used for processing and analysis of PET brain image data are well documented in the art.

The DeCarli methodology has been replaced by voxel based morphometric (VBM) measurements. VBM enables whole brain analyses on segmented images, and more precisely define where tissue loss occurs. This technology, using MRI, holds the potential for early discrimination; however, the problem with this method lies in the inherent variance in hippocampal volume, requiring successive measurements. Thus, two scans taken at least one year apart to get two volume measurements are needed to show a downward direction in volume greater than that seen in normal aging. One feature of the instant patent that provides an improvement over DeCarli and the methods developed using the DeCarli approach is the sensitivity to metabolic changes over a broad range of specific regions at a very early time point.

United States Patent 6,430,430, Gosche, relates to the use of MRI for the automated segmentation and volumetric measurement of white matter hyper-intensities, typically seen in multiple sclerosis (MS). Regions are defined by histogram intensity threshold-defined structures. This patent is similar to the DeCarli patent, as it is based on automated segmentation and thresholding algorithms. However the segmentation and volume measures go beyond individual brain structure and regions and also looks for specific and characteristic white-matter lesions found in MS. Because these lesions are hyperintense, they can be detected with relative ease by automated image segmenting and thresholding methods and their volumes subsequently measured. This could theoretically be applied to any such lesion with a significant intensity difference, either naturally or by the injection of contrast material to enhance visualization of the lesion, although this does not appear to be mentioned in this patent. The method taught in the Gosche patent to determine disease presence by searching for differences in volume and/or hyperintensity is essentially an extension and refinement of the teaching in the DeCarli patent. The Gosche patent teaches monitoring of disease progression with volumetric/intensity changes and could be useful to determine volume of various regions, detection of MS lesions and other disorders, such as AD, that induce volumetric or intensity changes. In contrast, the instant invention uses PET to measure metabolic changes in the previously described VOI's based on a volume radius as small or smaller than what might be seen in a MS lesion advanced enough to trigger the Gosche patent's hyperintensity threshold. The instant invention determines impairment by identifying differences in CDI values that occur early in the disease progression and monitors disease progression by tracking metabolic changes and the concomitant changes in the CDI, showing the utility of the instant invention for detecting MCI arising from a broad class of disorders, at an earlier stage of development with greater opportunity for the patient to obtain earlier, beneficial intervention.

United States Patent 6,374,130, Reiman, relates to the determination of radioactive glucose (FDG) uptake in the posterior cingulate region of transgenic mice. These mice have been transfected with a human gene that increases risk for AD, and have been treated with various agents which may have potential for treatment of AD. The posterior cingulate region in the brain is recognized as a region where metabolic changes have a high degree of sensitivity in the detection of AD. Mice have metabolic decline in this area related to the onset of pathophysiological changes brought on by the

transfected gene. This then becomes a model for the detection of agents that have the potential to treat AD, in that an agent which prevents or slows down the metabolic decline in the posterior cingulate region may prevent or slow down AD. Said agent could therefore be used in clinical trials in humans.

5 In contrast, the instant invention utilizes PET measurements of glucose metabolism in a plurality of three-dimensional volumes determined by SPM maxima-defined locations. The SPM techniques are thus applied more broadly and reveal those volumes exhibiting the greatest intensity change. The method of the instant invention is not restricted to only one region as is the Reiman patent. The method of the instant
10 invention is used to monitor disease progression by searching for differences in CDI values. These changes, subtle in the onset of disease, are detected at an earlier stage in humans and correlated with psychological and other tests of mental acuity. The method of the instant invention are, therefore, useful for detecting the changes in MCI resulting from the onset of AD, other degenerative diseases or injury to the brain. While the
15 posterior cingulate region is one of the most sensitive to the degradative onslaught of AD or similar diseases, it is not the only region that shows change. Further, the instant method is applied to determine MCI in closed head injury causing trauma to other regions of the brain than are customarily investigated in degenerative disease. Given the variation in the human population, the SPM overlay used to identify regions of maximal
20 change is clearly superior.

United States Patent Nos. 5,632,276 and 5,873,823, Eidelberg, et al., address the use of PET measurements of glucose metabolism in Parkinson's disease (PD). The patents recite possible application of the methods in AD, but include no description of any work done with patients suffering from AD. These patents teach use of a Scaled
25 Sub-profile Model (SSM), and (with the below-cited references, collectively, Eidelberg) describe how PET brain image data is obtained and stored digitally on a computer. The image is transformed into standardized stereotactic space by what is described as a "resizing and reorienting" procedure. The processed image is spatially filtered and patient scan images are then sampled in various regions, and the sampled data are
30 entered into an analysis. The analysis generates a "patient profile," which purportedly can be used to diagnose and discriminate this patient from other patient populations. The pattern of metabolic covariance (a subtype of factor analysis of variance, or FANOVA) within those regions is used to predict or indicate the presence or absence of

PD. Reported sensitivity for this method is 75 – 95 percent (Eidelberg et al., Early differential diagnosis of Parkinson's disease with 18F-fluorodeoxyglucose and positron emission tomography, *Neurology*, 45(11):1995-2004 (1995), Eidelberg et al., Assessment of disease severity in parkinsonism with 18F-fluorodeoxyglucose and PET, *J. Nucl. Med.* 36(3):378-83, (1995).

There are several significant differences between the method described in Eidelberg and the instant invention. Eidelberg and the instant invention both use FDG PET scans, collect PET brain image data, digitize the images, store digitized images on a computer, use spatial filtering methods (known in the art), sample data from various regions in the brain, calculate a number(s) based on the sampled data, and use the results to predict or diagnose neuropsychiatric illnesses. However, the differences between Eidelberg and the instant invention begin with the very application of the PET scan processing. The actual steps used to generate an FDG PET brain image are complex. Image data can be collected in 2D mode or 3D mode, reconstruction can be one of several major different types, and attenuation can be one of several types as well. Attenuation correction is very important as the signal change through the depth of the brain can be affected, and hence signal-to-noise can be altered. The instant invention is based on data showing that both reconstruction and attenuation correction algorithms affect the sensitivity of the CDI. No mention of any image collection, reconstruction, or attenuation correction routines is made in the Eidelberg patents. Further, standardization of an image in 3D space is done in a number of ways. There are three axes, and at least four linear warps that can take place along those axes (translation, rotation, scale, and shear). Typical routines use a least squares approach that minimizes the difference between a parent image and a target image with 12 parameters, and this is the method used in the instant patent. The "resized and reoriented" processing is not well defined by Eidelberg. Resizing could mean scaling, or it could simply mean removing extraneous space outside the brain image, and translating the image to a central standard location in the image space. Reorienting refers to rotation along the three axes. Thus, Eidelberg likely refers to a 6-parameter transformation, but at most, 9-parameters. This step is important as the definition of volumes of interest for the CDI are based on spatially standardized (12-parameter) images, not just co-registered (6-parameter) images. Also, it is unclear exactly which brain regions Eidelberg samples. Regions described in the earlier published literature of Eidelberg do not appear to be 1 cm

diameter spheres, and thus it is unclear whether they are the same regions, and they still are not clearly defined as to location. By contrast, the instant invention uses regions that are clearly defined (based on the results from objective statistical analyses) as being the most significantly different across the group analysis (specifically, VOIs based on SPM
5 maxima-defined locations as the center of 1 cm diameter spherical (0.5 cm³) volumes).

The mathematical methodology that creates the profiles in Eidelberg is completely different than the one used in creating the CDI of the instant invention. The CDI, as explained below, is a simple formula, but a prerequisite is weighting of the VOI's based on a frequency analysis described herein. As seen in the description of the
10 instant invention below, the formula is defined as a ratio of the mean of four regions identified with increased metabolism to the mean of nine regions with decreased metabolism, and combined with standardizing the group grand mean to a value of one with a scale factor (normalization). Eidelberg determines the presence of disease by assessing abnormal SSM values. The instant invention determines the presence of
15 disease by assessing abnormal CDI values. The Eidelberg method could be utilized to monitor disease progression by observing SSM value changes. The instant invention enables monitoring of the progression of disease by following CDI changes based on changes in metabolism.

United States Patent 5,434,050, Maggio et al., deals with the use of a beta-amyloid peptide fragment as a label of diseased tissue, and covers both *in vitro* and *in vivo* use, although all examples are *in vitro*. Samples of diseased tissue (containing amyloid plaques) bind the peptide fragment due to the self-binding/polymerizing nature of amyloid in plaque formation. This property is leveraged in the ability of the peptide to label plaques in a given tissue. Maggio covers radio-labeled peptides, as well as a
20 plurality of other labeling methods. While PET is mentioned as a potential means of detection of radio-labeled peptide binding *in vivo*, no discussion or description of how this could be carried out is given. Maggio does not address the potential problem of the failure of the peptide to cross the blood-brain barrier. Where the instant invention utilizes PET, Maggio uses multiple labeling methods of the beta-amyloid peptide. The
25 instant invention is based on defining volumes of brain tissue to be examined based on metabolic activity observed in specific volumes indicated by statistical analysis (SPM) whereas Maggio does not teach the use of any specific region. The instant invention is used to determine the presence of disease by searching for differences in the
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mathematically derived CDI and does not depend on brain biopsies. Maggio determines the presence of disease *in vitro* by assessing amyloid binding obtained from brain tissue examined for amyloid plaques.

5 United States Patent 5,109,868, Smith et al, teaches use of both structural (CT or MRI) and functional (SPECT) imaging methods in detecting/diagnosing AD. It uses a measure of width of the medial temporal lobe nearest to the brainstem as a marker for disease presence, defined in three different ways: 25% less than average thickness of controls, ratio of 0.75:1 (patient/controls), and/or 11.5 mm or less in size. Smith further teaches use of this measure as a marker is a cerebral blood flow deficit in the temporal-
10 parietal cortex based on a clinical evaluation done by a nuclear medicine physician, grading the SPECT scans from 0 (no obvious lesion) to 3 (severe perfusion deficit crossing the cortical rim). These are rather primitive measures by today's standards. SPECT is still more commonly used than PET for detection of AD, mainly due to cost. However it should be noted that this study is for the detection of AD, not MCI. These
15 markers are unlikely to indicate any change in MCI.

Smith teaches measurements of structure—width of the medial temporal lobe nearest to the brainstem (CT/MRI) and cerebral blood flow (SPECT) as opposed to the measurements of metabolism of the instant patent. The single region for CT in Smith is based on a defined anatomical location (posterior medial temporal lobe) and the general
20 locale of the temporal-parietal cortex (SPECT). The instant invention is derived from specific locations defined by SPM. Smith teaches the determination of AD by assessing decreased width of the medial temporal lobe nearest to the brainstem and decreased perfusion. The method in the instant invention contemplates determination of degenerative diseases or injury by searching for differences in CDI values and is not
25 restricted to AD. The methods taught by Smith have the potential to monitor the later stages of AD progression by observing decline in both measures. The CDI of the instant invention monitors progression of disease based on change in the CDI, which is likely to discern the existence of AD or other degenerative disease at an early stage of development.

30 United States Patent 5,617,861, Ross, et al., refers to measurement of brain metabolites using nuclear magnetic resonance spectroscopy (MRS). Both MRS and MRI are performed with the same hardware. Different software components are used to achieve the separate results. The spectra involved consist of a series of peaks, as

represented in Ross, which represent various chemicals: creatinine, N-acetyl aspartate, myo-inositol, and some others. These chemicals are metabolites of cell function. The essential feature of MRS is that these peaks change in various ways with various disease states. To measure changes in these metabolites, one begins by identifying a particular
5 volume element (voxel) on the brain image, and utilizes the software to collect data from that region. A voxel as defined for MRS is quite different than a voxel (elemental volume element in a 3D image) used elsewhere. The MRS voxel is essentially a region of interest. This typically encompasses 10 cm^3 , a rather large volume of brain tissue. The size of the voxel is directly related to the amount of time the subject being tested
10 must be scanned to obtain spectra of a given quality.

In contrast to the method of the instant invention measuring glucose metabolism, Ross teaches measurement of specific brain metabolites, myo-inositol, creatine and N-acetylaspartate. In Ross, the volume in which the metabolites are measured may be as large as 10 cm^3 in the medial temporal lobe or the posterior cingulate region. This is in
15 contrast to a volume of 0.51 cm^3 in SPM maxima-defined locations in the method of the instant invention. The larger volume in Ross is required by the mechanics of the measurement process rather than by the localization of precisely where in the region being examined the degenerative process causing cognitive decline occurs. In fact, the use of the larger volume in Ross, while it may include the diseased region, tends to
20 diminish the sensitivity of the technique because the values of the metabolite changes in the diseased region are averaged in with the more normal values obtained over the remainder of the voxel.

For the foregoing reasons, there is a need for a non-invasive, early stage method to obtain quantitative measures of mild cognitive impairment useful in diagnosing and
25 following degenerative brain disease or closed head injuries. The methods taught herein address this need by using *in situ* analysis of glucose metabolism in the brain using positron emission tomography, analyzing and transforming the image data, and using the data to construct a cognitive decline index (CDI) to measure mild cognitive impairment (MCI) indicative of the consequence of degenerative brain diseases or
30 traumatic, closed head injuries.

It is therefore the object of the invention to address the need for a non-invasive, early stage method to obtain quantitative measures of mild cognitive impairment useful in diagnosing and following degenerative brain disease or closed head injuries by

utilizing the image data from individual patient positron emission tomographic scans to construct a cognitive decline index which can serve as a diagnostic and screening tool to reveal the onset of mild cognitive impairment and nervous system dysfunction which are sequelae of degenerative brain diseases and closed head injury.

5 It is a further object of the invention to provide a method for determining the severity of said brain diseases or injuries.

 It is a further object of the invention to use successive measurements over a period of time to track the progression of degenerative brain disease in individual patients.

10 Still another object of the invention is to provide a method for producing an index indicative of brain disease comprising the steps of collecting positron emission tomographic image data showing metabolic activity in the brain of a patient, spatially normalizing said image data using a standardized three dimensional coordinate system, and spatially filtering the normalized image data. Specific regions of the brain showing
15 extremes in metabolic activity are selected and mean intensity values are collected for the normalized, filtered image data from said selected specific brain regions. The mean intensity values are weighted with standard weights derived from the group analysis used to create the standard and the ratio of the mean, weighted, metabolic activity image data are normalized to produce a numerical index.

20 It is a further object of the invention to assemble and maintain a data base of findings from individual patients which can provide reference points for comparison in the ongoing effort to understand and treat these diseases and conditions.

 It is a further object of the invention to monitor the effect of treatment of brain diseases and injury to quantify the effect of said treatment in ameliorating the disease or
25 injury.

 It is a further object of the invention to utilize the data from specific regions of the brain with appropriate weights in an artificial neural network to produce a Cognitive Decline Index with additional flexibility for applications to other diseases and conditions involving neural deficits.

30 It is a further object of the invention to utilize the capability of artificial neural networks to adapt to and capitalize on increases in a clinical data base reflecting experience with new patients.

The method of the instant invention provides these objects by enabling early detection of Mild Cognitive Impairment, a prodrome to Alzheimer's Disease. The methodological components include the specific location of the brain volumes of interest (VOIs) along with specific weighting factors derived from comparison of patient data and normal controls, and the creation of the normalized CDI from the mean weighted VOIs. Spatial normalization and filtering of a given image is coded simply, without having to use MATLAB or SPM, and a one cm diameter spherical VOI at each coordinate is sampled without the use of MARSBAR, as these are fairly straightforward mathematical algorithms. In one embodiment, the instant invention comprises a set of software routines that independently provide a CDI value for an appropriately processed FDG PET scan of a patient's brain. In a second embodiment, the method of the instant invention is extended using the data and processed information in the CDI to improve the results using a neural network approach to construct an additional CDI (CDInn). The high sensitivity of the CDI enables its use as a screening tool for the early detection of a variety of cognitive disorders.

Data from experiments performed to develop the instant invention show that analyses of groups of pre-morbid individuals can be discriminated from groups of normal controls. To date, there is no reliable method of detecting MCI in the early stages, at the clinical level, in individual patients. The development of the methods taught in the instant invention is overdue for clinical use to put this early detection capability into the hands of clinicians. Detection of the degenerative processes in pre-morbid or very early stages of brain degenerative disease, drug abuse or immediately following brain injury would enable the early treatment with medication to enhance and prolong quality of life, to provide an answer to the patients' questions regarding their potential for cognitive decline, to help them plan and prepare for the future, and hopefully one day, to prevent these diseases altogether or provide increasingly effective rehabilitative measures for injury.

In another aspect, the present invention comprises an apparatus for producing an index indicative of brain pathology resulting from injury, disease, or other cause. The apparatus includes a positron emission tomographic scanner and a computer operatively connected to the outputs of said PET scanner. The computer is programmed to calculate an index indicative of brain disease by:

collecting positron emission tomographic image data showing metabolic activity in the brain of a patient;

spatially normalizing the image data using a standardized three dimensional coordinate system;

5 spatially filtering the normalized image data;

selecting specific regions of the brain showing extremes in metabolic activity;

collecting mean intensity values for the normalized, filtered image data from the selected specific brain regions;

10 weighting the mean intensity values with standard weights derived from the group analysis used to create the standard; and

normalizing the ratio of the mean, weighted, metabolic activity image data to produce a numerical index.

In another embodiment of the invention, the CDI constructed in accordance with
15 the instant invention is repeated at time intervals in a process for monitoring the progress of treatment of cognitive impairment.

In another embodiment of the invention, the method is extended by utilizing the data in a neural network to further quantify mild cognitive impairment by constructing a Cognitive Decline Index using a trained neural network (CDInn). Neural networks are
20 composed of simple elements operating in parallel. As noted, these elements are inspired by biological nervous systems. As in nature, the network function is determined largely by the connections between elements. Neural networks can be trained to perform a particular function by adjusting the values of the connections (weights) between elements. Neural networks are especially relevant in situations
25 where a mathematical solution may be difficult or not optimal, or where there are numerous potential, example datasets from which a pattern or shape can be discerned. Commonly neural networks are adjusted, or trained, so that a particular input leads to a specific target output. Neural network nodes (neurons) are highly interconnected. They are designed to carry out pattern recognition or data classification processes. The
30 direction, number, and strength of connections between nodes determine how the network functions.

As stated, artificial neurons are based on simple abstraction of real brain neurons. A typical artificial neuron will have (as illustrated in FIG. 13, 14 and 16):

- inputs, numeric values,
- weights, also numeric values,
- a bias, a numeric value,
- an output, a numeric value,

5 and a transfer function. The output can be calculated by multiplying each input by its connection weight, adding them all together with a bias. This sum is passed to the neuron's transfer function. The function's output is the neurons output. The usual forms of the sigmoidal transfer functions used are tansig and logsig:

$$\text{tansig}(x) = [2 / (1 + e^{-2x})] - 1$$

10 $\text{logsig}(x) = 1 / (1 + e^{-x})$

Occasionally, the linear transfer function is used in back-propagation networks:

$$\text{Purelin}(x) = ax$$

where a is the slope of the linear function passing through (0,0).

The tansig is recognized as the hyperbolic tangent (sometimes written $\tanh(x)$).

15 The tansig function can be written in a more general form as:

$$\text{Tansig}(x) = (1 - e^{-\lambda x}) / (1 + e^{-\lambda x})$$

$$\text{Where } 0 < \lambda < \infty$$

It will be seen that tansig is a standard 2nd order curve with ranges from -1 to +1 and tansig = 0 when x = 0. These functions give the neuron a range of possible output
20 values. The bias is used to shift the input to the transfer function along the x-axis. The parameter λ is utilized to improve the efficiency of iterative training in the utilization of the neural network.

Different types of problems are handled by different network architectures. The most common artificial neural network is the Multi-Layer Perceptron (MLP) as shown
25 in FIG. 12. This particular neural network is a regular, feed-forward network.

Once trained, a neural network works as a function approximator or classifier. A neural network with an appropriate architecture can approximate any general function to arbitrary accuracy. Neural networks generalise well, that is they produce sensible outputs when presented with previously unseen inputs.

30 In another embodiment of the invention, the method is used for diagnosing Parkinson's Disease by measuring functional activity in a patient's brain and using the method to quantify mild cognitive impairment by constructing a cognitive decline index. The CDI and CDInn is correlated with other measures of mental acuity.

In another embodiment of the invention, the method is used for determining the severity of cognitive impairment by measuring functional activity in a patient's brain, using the method of the instant invention to construct a cognitive decline index or a neural network based cognitive decline index and correlating either or both indices with other measures of mental acuity.

Referring now to the figures, Figure 1 is a flow chart of the steps utilized in composing a database of normal control subjects and selection of patients for the application of the Cognitive Decline Index (CDI)

Figures 2A, 2B, 2C, and 2D are flow charts of the steps required to practice the preferred embodiment of the instant invention.

Figure 3 shows a PET scanner in accordance with the instant invention (FIG. 3A) and FDG PET scans of a normal subject control subject and a patient with Alzheimer's disease depicting enlarged regions of hypo-metabolism in the bilateral parietal and posterior cingulate region in the patient's brain as well as a more prominent motor strip as compared with the normal subject (FIG. 3B).

Figure 4 depicts Statistical Parametric Mapping (SPM) results showing decreases in brain metabolism in patients with early cognitive impairment compared to controls in a group analysis. Results are displayed in neurological orientation (images left is subject's left). The top left display is a maximum-intensity projection (MIP) image, AKA "glass-brain" image. This display shows all the voxels that were significant at the threshold chosen for display. For the purposes of this display, that threshold was fixed at a T of 2.5, and cluster size of 50 voxels. The upper-right shows the design matrix for the SPM compare-groups analysis. Below that are the read-outs for coordinates, cluster-level, and voxel-level statistics. The most stringent is voxel-level (corrected), and the most relaxed is voxel-level (uncorrected). Below that are some descriptive parameters for the statistical analysis, and below that is another table of coordinates from the large cluster in the left parieto-temporal area. The main readout from the results (the table above) will only report the three main foci in a blob. If there is a large blob, as is the case with the one in question, it is possible to get a printout of all the maxima in that blob, as was done here.

Figure 5 depicts SPM results showing increases in brain metabolism in patients with early cognitive impairment.

Figures 6A and 6B depict a cross-section (a) and 3D rendering (b) of regions of metabolic decrease in brain metabolism in patients with mild cognitive impairment compared to the subset of normal controls. The numeric color scale represents the SPM(t) values.

Figures 7A and 7B depicts a cross-section (a) and 3D rendering (b) of regions of metabolic increase in brain metabolism in patients with mild cognitive impairment compared to normal controls. The numeric color scale represents the SPM(t) values.

Figure 8 shows (a) a frontal, (b) lateral and (c) coronal views of the images from a FDG PET scan with the size and location of a volume of interest (VOI) superimposed.

Figure 9 displays results for the CDI across four groups of subjects (controls, mild cognitive impairment patients from a pilot study, mild cognitive impairment patients identified retrospectively, and Alzheimer's patients identified retrospectively) utilizing the method of the instant invention.

Figure 10 shows the correlation of age vs. the CDI for the four groups of subjects recited in Figure 9.

Figure 11 shows the separation obtained when using externally determined weights and examining only the MCI patients and older controls.

Figure 12 shows the distinct separation obtained when comparing MCI patients to older controls using externally determined weights as they relate to age.

Figure 13 shows the general structure of an artificial neuron with inputs, weights for each input, bias function, transfer function and output.

Figure 14 shows a regular feed forward network.

Figure 15 represents a neural network that is utilized to advantage in connection with the instant invention.

Figure 16 represents CDI neural network classification by two-layer, two-neuron network with linear and tansig transfer functions.

The data, analysis, calculations and procedures forming the method of the instant invention were produced in studies of four groups of patients:

1. Control subjects from the normal control database,
2. Patients identified retrospectively with early cognitive decline who received negative PET workups,
3. Patients identified retrospectively with cognitive decline and with the pathognomonic changes of AD present on PET imaging,

4. Patients with MCI from the pilot study.

The data used as exemplars in the figures and tables are taken from these studies.

Referring first to FIG. 1, a database 10 is compiled for normal control subjects. The database of FDG brain scans from healthy control subjects was created to enable the objective examination of a variety of patients who presented for clinical evaluation of cerebral pathology. These subjects are physically examined and screened for neurological and psychiatric illness. Entry into the data base requires that subjects have normal or unremarkable MRI scans as well as negative cognitive tests on Folstein Mini Mental Status Examination (MMSE) > 28 (Folstein et al., 1975). The control subjects' data from FDG PET normal brain scans is validated by comparison with patients with cerebral lesions. The normal control subject database is then used for statistical comparison in the method described as part of the instant invention.

Referring to FIG. 1, patients are selected and screened 20. Patients are categorized by the symptoms and history presented at examination. There are four categories: 101 memory complaints, family history of Alzheimer's disease, ApoE4 positive or related reasons; 102 history of substance abuse, concern over possible brain damage or related reasons; 103 history of head injury, cognitive complaints, headaches, blurry vision or related reasons; and 104 family history of Parkinson's disease, movement disorder or related reasons. Standard Clinical Evaluation is performed on the presenting patient 110 including physical examination, baseline laboratory tests; computerized tomography or Magnetic Resonance Imaging and neuro-psychological testing including the MMSE as well as the clock-drawing test (Shulman et al., 1986, Kirby et al, 2001) to search for memory and visuo-spatial impairments. These tests were chosen based on established sensitivity demonstrated in the literature (Petersen et al., 2001, Chen et al., 2001).

Other causes of cognitive decline are ruled out 115. These include neoplasm, endocrine imbalance, infection, nutritional deficiency etc. The patient is referred to the PET Center for evaluation of Cognitive Decline Index 120.

Referring to FIG. 2A, the CDI is designed to work with FDG PET brain scans obtained from a patient in standard clinical fashion. Standard clinical FDG PET scanning procedures were employed. Patients are injected with 10 mCi of FDG through a peripheral intravenous line. 200 The patient rests quietly in a darkened room with eyes and ears open for one hour for tracer uptake before scanning is begun. Control subjects

were injected with 5 mCi ²⁰⁵Tl to decrease radioactive exposure, and the scan time for the emission scan is subsequently increased to 20 min. ²¹³Tl to obtain equivalent counts.

As shown in FIG. 3A, scanning is carried out on a PET scanner 207, such as a GE ADVANCE™ scanner, in 2D mode with septa in place for all scans as at step 210.

5 A 10-minute emission scan is obtained ²¹¹Tl (20 min for controls ²¹³Tl), as well as a 5-min transmission scan ²¹¹Tl, ²¹⁴Tl. CDI sensitivity changes with variation in processing methods, and is optimized using reconstruction based on ordered subset expectation maximization (OSEM) ²¹⁵Tl and segmented transmission scan attenuation correction (SAC) ²²⁰Tl. In addition to standard clinical processing, a z-axis filtering step ²²⁵Tl is added to improve attenuation correction in the cerebrum and cerebellum on the base of the brain. Images are then reformatted for clinical evaluation on a computer operatively connected to the PET scanner 207 such as a Sun™ Ultra 60 ²³⁰Tl, and a copy prepared for export to the research workstation for use with SPM ²³⁵Tl (Friston et al, 1995a,b).

10 On the research workstation, scans are converted to Analyze 7.5™ (Analyze Direct, Lenexa, KS) format ²⁴⁰Tl. Initial image voxel resolution was 3.5 x 3.5 x 4.5 mm. These images are then further processed and analyzed with SPM99 (SPM, Friston et al, 1995a) implemented in MATLAB® (Mathworks, Natick, MA) ²⁴⁵Tl.

Facility in management of the image data is achieved by utilizing the Digital Imaging and Communication in Medicine (DICOM) format. ²⁵⁰Tl (see, <http://medical.nema.org>). The (DICOM) standard was created by the National Electrical Manufacturers Association (NEMA) to aid the distribution and viewing of medical images, such as CT scans, MRIs, and ultrasound. Additionally, the image data are further conditioned to enable data management on particular work stations ²⁶⁰Tl and ²⁶⁵Tl.

25 Once loaded into SPM, image data are spatially transformed based on a brain template into the standardized 3D space developed by the Montreal Neurological Institute (MNI) ²⁷⁰Tl. There is considerable variation in the size and shape of the human brain and standardization is needed to compare patients to normal controls, as well as achieving comparability between patients for the patient database. Standardization, and the resulting coordinate space is known as Talairach space, and was described by Talairach and Tournoux, 1988. Coordinates are transformed using mni2tal (Matthew Brett's mni2tal.m is found at <http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html>).

30 The method for standardization of image data to this space (spatial normalization) involves the use of twelve-parameter linear affine mathematical routines

to translate, rotate, scale, and shear the image along the X, Y, and Z axes (4 actions x 3 axes = 12 parameters). A template brain scan is used as a standard, and the brain scan being normalized is matched as closely as possible to the template in shape, size, and space. This has been well described previously (Friston et al, 1995a). Briefly, the brain image data is moved (translated) to the center of the image, twisted to match the orientation of the template (rotation), and scaled to best match the size of the template. The fourth step involves shear, such that one plane of the image slides on the next to optimally fit the shape of the object image to that of the target (template) image. All four steps are carried out along all three axes in 3D space. A final processing step to optimize across-subject analyses of PET image data is spatially transformed spatial filtering, also known as smoothing. This step increases the signal-to-noise ratio and decreases variance across the PET image data by removing much of the variability between patients due to differences in gyri/sulci patterns. This is necessary to achieve optimal comparability in the patient data base. The optimal smoothing level is generally agreed in the art to be approximately 1.5 to 2 times the full-width half-maximum parameter of the scanner, considered to be the spatial resolution. For this research, the GE Advance scanner 207 has a resolution of approximately 3.5mm x 3.5mm x 4.5mm. Spatial filtering with a Gaussian kernel (the standard method) also renders the data amenable to analysis via methods incorporating the theory of Gaussian fields, which is important for the majority of the statistical routines used by the SPM software package.

PET scanner 207 measures the energy from positron emission, and an image of brain function is created for the entire brain. An example is shown in FIG. 3B, with the image in the top panel (a) from a 65 year-old normal subject, and (b) is an FDG brain image from a 67 year-old patient with probable Alzheimer's dementia. This pattern is pathognomonic of AD. However the gold standard is still brain biopsy, with tissue diagnosis based on amyloid plaques and neurofibrillary tangles. It is notable that often the FDG brain scans from patients with MCI appear normal, just like the scan in (a), even to the well-trained nuclear medicine physician's eye. Thus, these scans are read clinically (subjectively) as not consistent with MCI or AD. Radiological evaluation of the MRI is even less sensitive, and often patients with moderate AD, like the one in (b) still have normal or clinically unremarkable MRIs.

The two brain scans in FIG. 3B are examples of scans that have been spatially normalized. This spatial transformation can be problematic in images that have large

lesions or severe atrophy, however in patients with lesions too subtle to detect clinically and/or mild atrophy there is very little error. Nevertheless, all brain images were inspected visually post-normalization to assess the quality of the transformation. One notable change from the standard is that all images were interpolated into the template spatial bounding box, instead of the standard bounding box. The template boundaries include the entire brain including the cerebellum, while the standard one cuts out the majority of this important brain component. After the spatial transformation, further residual inter-subject differences (due mainly to variation in patterns of gyri and sulci) were minimized by smoothing with a Gaussian filter kernel (8 mm isotropic). This also serves the purpose of ensuring the data are normally distributed; hence Gaussian Field theory can be applied in the analysis of the images (see below). No partial volume correction was carried out for any scans in either group, and there were some scans in both groups with mild generalized atrophy. A previous study examines partial volume correction as a means to correct for the effects of atrophy on the PET metabolic data (Meltzer et al, 1996). However, there were very few subjects with clinically detectable atrophy, the atrophy present was mild, and there were both MCI patients and controls with mild atrophy, so correction for partial volume was unnecessary. Further, another report finds that atrophy does not play a major role in PET metabolic data (Ibanez et al, 1998). Final image voxel size was 2 x 2 x 2 mm. These spatially normalized and smoothed images were then used in the SPM analyses to determine regions of significant difference in metabolism between the controls and patients.

Referring again to FIG. 2B, patient data is compared to controls of a similar age range 300 to identify extrema in increases and decreases in metabolic activity 310. The group analysis was carried out in SPM, a software package designed for processing and analysis of brain images. The mathematical requirements for the analyses of 3D images involve application of the General Linear Model and Gaussian Field Theory (Friston et al, 1995b; Worsley et al, 1995). The compare-groups statistical model is used in this analysis to produce a SPM(t) statistical map 320. This is then converted to the unit normal distribution Z score 330. Clusters with significant increased or decreased metabolic activity are then identified 340. A significance threshold uncorrected for multiple comparisons is used. This is supported by previous reports in the literature which indicated similar patterns of activity (De Leon et al, 2001; Reiman et al, 2001; Small et al, 2000) and by the analysis showing that many of the brain regions under

examination were identified a priori as being likely regions to have decreased metabolism. This analysis enables identification of brain regions for use in creation of the CDI. The locations of significant points of interest are determined from the SPM results 350 (see FIGS. 4 and 5 for examples of the features providing these results).

5 This printout of SPM results lists all maxima greater than 8mm apart. Coordinates from the main foci are used in the creation of VOIs 360 for the calculation of the CDI (see below). FIGS. 6 and 7 show the maps of significant differences overlaid on canonical brain images, in both cross-sectional (a) and 3D rendered (b) views. FIG. 6 shows regions of decreased metabolism, and FIG. 7 shows regions of increased metabolism.

10 Specific loci from this analysis are used as centers for 3-D, 1 cm diameter spherical (VOIs) created with the MARSBAR™ plug-in (Bret et al, 2002) for SPM 370. This size VOI is selected because it approximates the spatial resolution of the data post-smoothing. Those skilled in the art will recognize, however, that other volumes may be appropriate for use as the volume of interest depending upon such factors as scanner
15 resolution, patient tolerance of radio ligand, refinement of the statistical methods, size of both patient and normal subject database (the latter for comparison purposes as set out below), suspected disease/impairment, and other applicable factors. The intensity of each of the voxels within the spherical VOI is read and the average is obtained 380. Raw data uncorrected for global intensity differences are used, since a ratio created from
20 these data intrinsically corrects for differences across subjects.

In a first embodiment, mean image intensity values were collected from 13 regions for each subject. These regions are composed of areas that showed either increased metabolism (cerebellum, pons, sensorimotor) or decreased metabolism (temporal lobe, hippocampus, parietal lobe, frontal lobe, posterior cingulate). Two steps
25 for determination of weights for each VOI are used. The first set of weights for each VOI are based on the frequency of abnormality of the VOI data from all study patients as compared to all controls, with higher weights applied for increasing frequency 390. The CDI calculated with these weights is designated CDI_1 . These weights are then used as a baseline to calculate a second CDI (CDI_2) involving iterative optimization of each
30 weight to maximally separate patient from controls according to observer criteria 395. For both CDI_1 and CDI_2 steps, the global mean of the weighted control group VOI ratio is normalized to a value of 1, and this normalization factor then universally applied. The resulting, mean, weighted, normalized VOI ratio forms the CDI.

Once CDI values were obtained for each patient, the normal distribution of the data was established with a Kolmogorov-Smirnov statistic. Groups were analyzed for significant differences with analysis of variance, and the confidence level was determined for each group. Two-tailed t-tests were applied to establish the significant differences between the groups of subjects. The analysis performed in the exemplar study determined weights for each VOI. With the method completed and validated, the weights thus produced are used for each new patient that presents.

The MARSBAR SPM toolbox is a plug-in type program for SPM, and is used to create the 3D, one cm diameter, spherical volumes of interest (VOIs) used to sample data (Brett et al, 2002). MARSBAR produces a mean intensity value for the volume elements (voxels) present within the volume of interest. The voxels are cubes 2 mm on a side after spatial normalization and the spherical VOIs represent a bounded volume containing the voxel, and is thus not a perfect sphere. See FIG. 8, step 395, for an example of the location and representative size of an exemplar VOI. The CDI is derived from 13 VOIs located at specific coordinates, including specific locations in the parietal cortex, medial and lateral temporal areas, frontal cortex, and posterior cingulate cortex, as well as the sensorimotor cortex, cerebellum, and pons. There were initially more VOIs from the majority of the maxima presented in the tables from FIGS. 4 and 5, however it was discovered that several of the regions were not necessary. This was discovered by iteratively examining the CDI results with fewer and fewer VOIs. The use of 13 VOIs proved optimal, although 11 also worked well, and as set out below, in a second embodiment, the method of the instant invention is utilized to advantage with the intensity values from only nine regions to produce a CDI using a trainable neural network. The sensitivity, as judged by the separation of the patients from the control CDIs, dropped somewhat if fewer regions were used, and did not appreciably increase if more regions were used. Their exact locations in 3D space are given in Table 1:

Region	XYZ	CDI ₁	CDI ₂
	Coordinates	Weights	Weights
Increased activity (numerator)			
R pons	10 -22 -26	10	20.1
L vermis	-6 -54 -14	1	11.6
R cerebellar nuclei	14 -38 -34	7	18.8
L sensorimotor	-16 -24 52	7	30
Decreased activity (denominator)			

L post. cingulate	-4 -70 30	13	5.5
L frontal	-26 48 16	13	5.7
L parietal	-42 -74 36	10	4.3
1 st L temporal	-56 -44 -20	4	4.7
2 nd L temporal	-56 -56 16	8	1.8
L med temporal	-24 -12 -28	1	1
R parietal	54 -66 32	7	-2.5
Basal nucleus	-6 14 -20	5	-4
L post. hippocampus	-26 -36 -8	5	3

Table 1

Weighting factors and coordinates of regions used for development of the CDI. CDI₁ weights were derived as described in the test from examination of the frequency of abnormalities in the patient group, while CDI₂ weights were derived arbitrarily to optimize the separation between the two groups. This is why the image must be spatially normalized, to ensure that the VOIs are sampled at exact coordinates determined by the SPM analysis to be sensitive to the metabolic changes of MCI, in the same spot in every subject. Mean image intensity data was sampled for all 13 VOIs in all subjects. Data for each VOI and patient is displayed in MATLAB® and saved to a text file for further processing and analysis. Uncorrected (raw) image intensity data was sampled because any global intensity correction or scaling at this point is unnecessary because a ratio of some VOIs to others from the same image will be obtained, and this automatically and intrinsically corrects for global inter-subject intensity differences. These VOIs formed the raw data for the creation of the CDI.

The VOI ratio without weights has good performance in separating patients from controls. This ratio is determined by obtaining the mean, \bar{X} , of the four VOIs from regions with increased metabolism (defined here as X₁ through X₄), the mean, \bar{Y} , of the nine VOIs from regions with decreased metabolism (Y₁ through Y₉) and dividing the mean of increases by the mean of decreases, \bar{X}/\bar{Y} . The results of this calculation for the above referenced study are shown in Table 2:

	Controls	MCI	AD
Mean	0.639	0.779	0.928
SD	0.034	0.063	0.127
Minimum	0.577	0.697	0.709
Maximum	0.713	0.915	1.185
Count	33	17	15
95% CL	0.012	0.032	0.070

Table 2. Results from analysis of VOI ratios, without weighting normalizing. Note overlap between Controls and MCI.

The first embodiment of the instant invention is a sensitive indicator and discriminator. The first embodiment is obtained by determining and applying weighting factors to each

5 VOI. Multiple mechanisms were evaluated for determining and assessing appropriate weights. In the second embodiment, an approach using weighted intensity values as inputs to a trainable neural network resulted in even greater discrimination in the CDI that was produced. In the method used in this first embodiment, weights for each VOI were based on frequency of abnormality of that VOI across all patients and all controls,

10 with higher weights applied for increasing frequency (the weighting process utilized in connection with the second embodiment is set out below). The CDI calculated with these weights is designated CDI_1 . For the frequency analysis, the un-weighted VOI values are used to generate two VOI ratio datasets. The first dataset is composed of nine VOI ratios formed by dividing the mean of the four increases, \bar{X} , by each of the

15 decreases; namely, $R_i = \bar{X} / Y_i$ for $1 \leq i \leq 9$. The second dataset is composed of 4 VOI ratios formed by dividing each of the increases by the mean of the decreases; namely $R_j = X_j / \bar{Y}$ for $1 \leq j \leq 4$. In the exemplar study, each of the 13 VOI ratios for each patient was compared to the controls to assess the degree of overlap in the patient vs. controls there was in the data ranges. Separate weights were calculated for the

20 numerator and denominator VOIs. For example, for the VOIs from the posterior cingulate at [-4, -70, 30], the VOI range for all patients was 0.869-1.895, and the control range was 1.988-2.705. For the patients, 29/32 VOIs were outside the range for the normal group. The VOI with the lowest number falling outside the normal range was at [-24, -12, -28] (left medial temporal), with 17/32. This was set to one by subtracting 16,

25 which was also subtracted from all the weights for other VOIs in the denominator, thus leaving a range for individual weight values, designated as W_i , from 1 for left temporal to 13 for posterior cingulate. A similar process was carried out for weights for VOIs in the numerator, resulting in individual weights, designated V_j , for both components of the ratio, shown in Tables 2 and 3. Once the weights were generated and applied, the

30 weighted VOIs were used to calculate the CDI for the study patients. The weighted VOI ratio was then normalized, such that the grand mean of the VOI ratios from the control

group was set to one, and the resulting correction factor was then applied to all weighted VOI ratios. The mean, weighted, normalized VOI ratio constitutes the CDI_1 400.

$$CDI = C_x + \left[\sum_{j=1}^n V_j X_j / n \right] / \left[\sum_{i=1}^m W_i Y_i / m \right]$$

- 5 Where, X_j denotes the j^{th} Increased Intensity Value;
 V_j denotes the j^{th} Weight for the j^{th} Increased Intensity Value;
 Y_i denotes the i^{th} Decreased Intensity Value; and
 W_i denotes the i^{th} Weight for the i^{th} Decreased Intensity Value.
 C_x is the correction factor used to normalize the dataset

- 10 The weights have been established with $n = 4$ and $m = 9$. Once established, this set of weights is used for each new patient presenting for scanning and diagnosis.

The set of steps for calculating CDI_1 are: (1) Import VOI Data Into Spreadsheet 391; (2) Determine Intensity Range Overlap for each VOI Ratio 392; (3) Create Weights for each Intensity Extreme 393; (4) Create Weighted VOI Ratio 394; and (5) Scale and
 15 Normalize Ratio 395.

Demographic and screening information are presented in Table 3 for the subject groups used in the referenced exemplar study.

	N	F/M	Age (Mean+/- SD)		Min	Max
Controls	33	10/1 7	51.2	17.7	19	81
Old controls	19	10/9	63.9	9.3	51	81
MCI-pros	5	3/2	73.0	7.7	64	85
MCI-retro	12	6/11	68.2	6.4	52	76
AD	15	6/9	66.5	9.2	53	80

Table 3

- 20 It was not possible in the study to determine the MMSE scores of all patients identified retrospectively. The group of older controls was used for the SPM analysis, but all controls were included in the CDI results for comparative purposes. For the MCI patients from the pilot study, the mean MMSE was 25.3 ± 2 , and the CDT was 3.3 ± 0.8 .
 25 For the older subset of controls used in the SPM analysis, the mean MMSE was 29.3 ± 0.8 , and the mean CDT was 3.8 ± 0.4 .

The results of the SPM group analysis of the patients with MCI vs. controls are shown in FIGS. 4 and 5. The retrospective and prospective MCI scan datasets were

pooled for this evaluation. Patients were compared to a subset of controls matched for age. FIG. 3 shows the regions of decreased cerebral metabolism that were present in the group analysis. These included many regions characteristic of that seen in previous studies of MCI and AD, including the basal nucleus region, posterior cingulate, bilateral parietal, several left temporal and hippocampus regions, and left frontal regions were found. FIG. 4 shows the regions of increased metabolism in patients with MCI. This includes regions in bilateral motor areas, cerebellum, pons, and a right parietal area that is more medial and superior to the regions found with decreased metabolism. FIGS. 5 and 6 show the maps of significant differences overlaid on canonical brain images, in both cross-sectional (a.) and 3D rendered (b.) views. FIG. 5 shows regions of decreased metabolism, and FIG. 6 shows regions of increased metabolism.

FIG. 8 shows an example of one VOI (posterior cingulate). Data was sampled for VOIs from all 13 regions to calculate the CDI as described above. A comparison of the grouped CDI₁ values was carried out, and the results are shown in Table 4.

	Controls	MCI(all)	AD
Mean	1.000	1.112	1.224
SD	0.027	0.046	0.094
Minimum	0.949	1.051	1.076
Maximum	1.042	1.222	1.414
Count	33	17	15
95% CL	0.010	0.024	0.052

Table 4

As it was unclear whether these data were normally distributed, a Kolmogorov-Smirnov test was carried out and indicated a normal distribution of the data (Table 5):

Kolmogorov-Smirnov	Controls	MCI-pros	MCI-retro	AD
N	33	5	12	15
Mean	1.000	1.114	1.111	1.223
SD	.026	.048	.047	.094
Absolute	.096	.241	.161	.082
Positive	.059	.236	.161	.074
Negative	-.096	-.241	-.100	-.082
KS Z-score	.549	.540	.558	.317
p value (2-tailed)	.924	.933	.915	1.000

Table 5

Tables 6 (ANOVA) and 7 (t-tests between groups) show statistical analyses for significance:

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.538	3	0.179	60.020	3.44E-18	2.755
Within Groups	0.182	61	0.003			
Total	0.720	64				

Table 6

Group t-tests	Control	MCI-pros	Control	MCI-retro	Control	AD	MCI-pros	MCI-retro
Mean	1.000	1.114	1.000	1.111	1.000	1.224	1.114	1.111
Variance	0.001	0.002	0.001	0.002	0.001	0.009	0.002	0.002
Observations	33	5	33	12	33	15	5	12
Df	36		43		46		15	
T Stat	7.856		9.901		12.653		0.114	
P(T<=t) two-tail	2.56E-09		1.17E-12		1.379E-16		0.910563	
T Critical two-tail	2.028		2.017		2.013		2.131	

Table 7

5 All patient groups were highly significant compared to controls, but there was no difference between patients with early cognitive decline identified retrospectively and those obtained from the pilot MCI study. The normal range for this study was 0.949 to 1.042, (95% CI 0.990 – 1.010). This critical data range is the embodiment of the normal standard range to which all patient CDI values are compared. The CDI₁ was successful in discriminating 100% of the MCI patients in both the retrospectively and prospectively identified groups (range 1.051 – 1.222, 95% CI 1.088 – 1.136), as well as all probable AD patients (range 1.076 – 1.414, 95% CI 1.172 – 1.276). Separation of patients with MCI from controls is shown in FIG. 9, which also shows results for the AD group for comparison. The lack of a relationship of the CDI to age is shown in FIG. 10.

The initial weights of the intensity values were used as a baseline for calculating a second CDI (CDI₂) involving iterative optimization of each weight to maximally

separate study patients from controls in neural-network fashion. A dynamic table was created where the results of a change of a given weight upon the separation of the groups could be assessed in real-time. Weights were iteratively adjusted to maximize separation between the control and MCI populations while minimizing within-group variance. Using this arbitrary method, the weights resulting in optimal separation between the two populations were determined, and are shown in Tables 8 and 9.

	Controls	MCI (all)	AD
Mean	1.000	4.595	5.659
SD	0.671	1.622	3.205
Minimum	-0.687	2.976	2.315
Maximum	2.237	7.376	13.252
Count	33	17	15
95% CL	0.238	0.834	1.775

Table 8

Group t-tests	Controls	MCI- pros	Controls	MCI- retro	Controls	AD	MCI- pros	MCI- retro
Mean	1.000	5.010	1.000	4.432	1.000	5.659	5.010	4.432
Variance	0.451	3.933	0.451	2.255	0.451	10.271	3.933	2.255
Observations	33	5	33	12	33	15	5	12
Df	36		43		46		15	
t Stat	-9.131		-10.662		-8.068		0.660	
P(T<=t) one-tail	3.33E-11		5.95E-14		1.17E-10		0.260	
t Critical one-tail	1.688		1.681		1.679		1.753	
P(T<=t) two-tail	6.66E-11		1.19E-13		2.35E-10		0.519	
t Critical two-tail	2.028		2.017		2.013		2.131	

Table 9

Results from the group analysis using CDI₂ are shown in Table 8, and are presented graphically in FIGS. 11 and 12. Statistical significance is presented in Table 9. While this method of CDI creation does result in the best discrimination of MCI patients from older controls, it did not discriminate patients with AD as well as CDI₁.

The SPM analysis discussed above is valuable, but has significant drawbacks. Determination of significance is somewhat arbitrary. The most conservative significance level is non-a-priori, based upon the intensity at the single-voxel level, and

typically requires an SPM(Z) statistic in the 4.5 to 5 range to be determined truly significant. The least conservative significance level is based on an a priori hypothesis about activity in a given region, examines spatial extent or a combination of extent and peak height more so than intensity, and can be as low as a Z score of perhaps 2.5. It was noted that a characteristic pattern of decreased metabolism could emerge if the significance level was set to low levels. This “trend” in characteristic patterns was what was seen in several patients with MCI, where there were no obvious clinically defined lesions. Because of the problematic statistical significance question involved, and because it can be difficult to interpret a pattern of activity, especially in the light of low thresholds, it was necessary to create a way of objective examination of the PET brain image data that is more definitive and usable on the single patient level. Because analysis of ROIs removes the major problem of multiple comparisons and conservative Bonferroni adjustments, this method was the main focus of further research beyond SPM and led to the methodology discussed in the preferred embodiment.

Using ROIs to examine both semi-quantitative and absolute brain image data was once the major methodology in use (and is still common), as the voxel-by-voxel approach incorporated in SPM is a relatively novel method. Most studies of semi-quantitative image data intensity normalize the data by dividing a given ROI value by that obtained from the pons (e.g., de Santi et al, 2001), the cerebellum (e.g., Cappa et al, 2001), some other “standard” (presumptively unaffected) region such as the sensorimotor cortex (e.g., Arnaiz et al, 2001), or by an estimate of the global value (de Leon et al, 2001). However, the cerebellum, pons, and sensorimotor area were all found to have increased activity in the SPM analysis when patients with MCI were compared to control subjects in the method of the instant invention. All these regions, as outlined above, have been used as reference regions in studies of AD in the belief that they are preserved and thus represent normal rates of metabolism. Alternatively, it is possible to look at the relationship between two regions on opposite sides of the brain by creating a ratio, as in the “asymmetry index” (Russel et al, 1997). One of the major problems of ROI analysis of functional imaging data (pre-SPM) is that definition of the shape, size, and location of the ROI is often subjective and arbitrary. There are many variations on this theme. In essence, using ROIs arbitrarily predefine a hypothetical lesion. For example, if an ROI 2 cm in size is arbitrarily placed in the temporal lobe to interrogate for a region of hypometabolism, and is positioned over a portion of a 1 cm lesion, then

the (averaged) intensity value from the ROI will have increased variance due to being the mean of voxels from outside the lesion that are averaged together with voxels from within the lesion. The end result may be an ROI value that lacks significance. The solution to this problem is to place ROIs in positions where there is known pathology, e.g., based on the results of an SPM analysis. ROIs have been previously derived from SPM regional maxima (Buchel and Friston, 1997). Thus, the use of ROIs in the instant invention, has evolved past the earlier usage. The employment of ROIs in the instant invention escapes the major problem inherent in previous of SPM analyses (multiple comparisons), and also escapes the main problem historically associated with ROI analysis of having an arbitrary location in relation to the suspected pathology.

The CDI was derived by examining the regions found to be significant, or trending towards significance, in SPM analysis. All regions used for the CDI were derived from the SPM analysis. While it may be possible to obtain an equally valid CDI with more or fewer regions, arriving at the 9 regions of decreased metabolism and 4 regions of increased metabolism was essentially arbitrary. These were the major regions that separated out of the SPM analysis of older controls vs. MCI patients. The number of regions used was derived selecting SPM-defined regions of maximal difference, and determining which of these regions had the highest degree of separation between the two groups (frequency analysis).

Referring now to the above-described second embodiment of the method of the instant invention (designated CDInn), the MATLAB® Neural Networking (NN) Toolbox (Mathworks, Inc) is a component of the MATLAB program. It allows for the construction of artificial neural networks in the MATLAB computer environment. The methodology for the CDInn involves the use of a simple feed-forward, back-propagation NN with 9 inputs and 2 layers as in FIG. 15. The preferred embodiment described in the instant application comprises a 2-neuron (one hidden, one output) network with linear then tangent-sigmoidal (tansig) transfer functions. In MATLAB, after ensuring that the Neural Network Toolbox has been added to the MATLAB path 600 (FIG. 2D), the command "nntool" is typed at the MATLAB command prompt to start the NN graphical user interface 610. A network is specified with the following parameters 620:

<ul style="list-style-type: none"> • Name: CDInn • Type: Feed-forward back-propagation • Input ranges: [-1 1; -1 1; -1 1; -1 1; -1 1; -1 1; -1 1; -1 1;] • Training function: trainlm • Adaption learning function: Learndgm • Performance function: MSE • Layers: 2 • Number of neurons: 2 • Transfer function neuron 1: purelin • Transfer function neuron 2: tansig 	<p>The CDInn is exemplified in FIG. 15</p>
--	--

VOI regions obtained from the specified locations were used to construct inputs to the neural network 630. In this second embodiment to construct the CDInn, nine (9) regions were used instead of the thirteen (13) locations for construction of the CDI.

5 Statistical analysis on group x region data utilized for construction of the CDI in the first embodiment of the instant invention indicated that four (4) regions offered no discrimination between patients and controls. Thus, these four regions did not add any significant influence on the function of the neural network and were therefore removed to give nine regions for the CDInn that were:

10 VOIs from regions of increased activity: vermis, motor, R pons, cerebellar nuclei
VOIs from regions of decreased activity: posterior cingulate, L parietal, R parietal, L temporal01, L temporal02.

The order of regions entered into the NN was:

15 vermis, motor, R pons, cerebellar nuclei, posterior cingulate, L parietal, L temporal01, L temporal02, R parietal 640.

VOI data are mean-normalized by subject 650, i.e. divided by the average of the 9 locations measured for each subject. This gave each subject's data set a mean value = 1. Datasets are then split into evenly divided subgroups 660 (c1 denotes control subgroup # 1, p1 = patient subgroup #1, etc): [c1=16, c2=17, p1=16, p2=16]. To provide the
20 neural network small datasets with mixtures of patients and controls for training, four mixed groups are made by pairing controls with patients to give: [c1p1, c1p2, c2p1, c2p2] 670. These datasets are then exported (from EXCEL™) to a text file 680,

imported into MATLAB, and converted to a .mat file for use by MATLAB. Rescaling
 690 is then done to ensure the input range is appropriately matched for the tansig
 function on the output layer of the NN, which will classify the input data in the range \pm
 1. This is carried out in the main MATLAB window at the command prompt. As an
 5 example, the dataset c1p1 is rescaled using the premnmx function:

```
[c1p1_rescale,minp,maxp]=premnmx(c1p1)
```

The rescaled output from this function is labeled 700 and saved as c1p1_rescale.mat.
 This procedure is performed for each of the four paired datasets listed above. These
 four files are then used as "inputs" for neural net training 710. For training of the neural
 10 net, each input file has a "target" file to which the neural network matches the input
 dataset. These files are labeled to match the dataset from which they originated, thus for
 c1p1_rescale.mat, the "target" file is labeled c1p1t.mat. Each target file consists of a
 single row of digits with the same number of columns as the input file. For each patient
 (column) in the c1p1_rescale dataset, a "-1" is present in the c1p1t.mat file, and for each
 15 control, a "1" is assigned. Thus, the four input and target datasets are:

<u>Input</u>	<u>Target</u>
c1p1_rescale.mat	c1p1t.mat
c1p2_rescale.mat	c1p2t.mat
c2p1_rescale.mat	c2p1t.mat
20 c2p2_rescale.mat	c2p2t.mat

The network is then trained on each input dataset 720, and then the resulting trained
 CDInn was tested on the full dataset to assess its classification accuracy 730. Output
 from the 2-layer, 2-neuron neural network on 65 subjects (33 Cons, 32 AD) derived
 from mean-normalized and scaled measures from nine PET scan regions.

25 The network trained on c1p1 misclassified one control from c2 and one patient
 from p2. Thus, this simple network trains very quickly. However after further training
 on c1p2, c2p1, and c2p2, the network achieved 100% accuracy in its classification of
 patients and controls. The output of the network (1 = normal, -1 = abnormal) is used to
 determine whether a patient has MCI or not.

Training error = [7.2555e-009 1.1596e-006 0 0 0 6.9162e-010 9.5137e-011 0 0 2.6645e-015 0 0 0 0 0 0 -2.0697e-008 0 0 0 -9.9476e-014 0 0 0 0 0 0 -1.0384e-008 0 -5.3459e-008 0 -1.0129e-008]

```
5    Tsum = 1.0730e-006
```

Test output = [1 -1 -1 -1 -1 -
1 -1]

Classification = [1 -1 -1 -1 -1
-1 -1]

$$\text{MSE} = 0$$

15 Example 4. C2P2

Weight to layer 1 from input 1 = [-2.6405 -6.9911 -11.9418 8.8868 4.3081 0.36847 -0.51791 -1.7387 4.2559]

20 Weight to layer 2 from output 1 = [4.9862]

Bias to layer 1 = [-1.2351]

Bias to layer 2 = [-4.9036]

25

Training error = [8.8818e-016 0 9.3856e-007 0 0 0 8.4302e-008 0 0 0 0 0 1.8353e-009
0 0 0 0 0 0 -6.3943e-009 0 0 -5.8602e-010 0 0 -3.8585e-010 -4.4476e-013 -9.0324e-011
0 0 0]

```
30    Tsum = 1.0172e-006
```

Test output ALL data = [-1 0.9998 -1 -1 -1 -0.99997 -1 -1 0.97991 -1 -1 -1 -0.99999 -1
0.98121 -0.94774 1 -1 -1 -1 1 -1 -1 0.9998 -1 -1 0.99989 0.98143 0.63469 -1 -1 -1 1 1 1
0.99898 1 1 1 1 1 1 1 1 0.99941 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1]

35

Classification = [-1
-1 -1 -1 -1 -1 -1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1]

$$\text{MSE} = 0$$

40

The CDI of the instant invention is constructed using weights based upon the frequency of intensity abnormalities found in thirteen (13) regions identified as described above. Whereas most ratios are between one region under examination and another region used as a standard, the ratio in the CDI is derived from the mean of four of the weighted ROIs divided by the mean of the other nine weighted ROIs. All ROIs are being examined experimentally; there are none that are arbitrarily chosen as the

“standard” region or regions. This use of ROIs has not been taught previously in the art. Thus, forming a mean for the numerator and denominator is novel, derivation of the weights is novel, and using ROIs from increases for the numerator and ROIs from decreases for the denominator is novel. Forming a mean, weighted, normalized ratio is thus a unique approach in the detection of MCI. While several of the regions derived from the SPM analysis are consistent with those reported in the literature as being involved in MCI/AD pathophysiology (e.g., NBM, medial temporal, posterior cingulate, superior parietal) several of them, especially regions of increased activity, have not previously been reported, and are thus essentially novel to this method. Moreover, while there have been anecdotal reports of sensorimotor cortex preservation in AD (Arnaiz et al, 2001), no one has previously reported increased activity in this region from an SPM analysis, related to MCI.

The CDInn of the instant invention is based on the analysis of intensity values from nine (9) regions in subject brains. These regions as specified above, namely, the vermis, motor, R pons, cerebellar nuclei, posterior cingulate, L parietal, R parietal, L temporal 01, and L temporal02, showed statistically significant changes in intensity. The changes in these regions are consistent across subjects that were found to exhibit MCI and found to discriminate between subjects and controls. Reduction of the original thirteen (13) regions of increased or decreased intensity as used in constructing the CDI to the nine (9) regions used to construct the CDInn form the basis of optimizing the initial neuronal set of the neural network.

It has become apparent from many studies appearing in the functional imaging literature that the cerebellum often plays a major role in cognitive as well as it's more well known motor functions (Parsons et al, 1997; Rapoport et al, 2000). The neo-cerebellum has undergone striking parallel evolution with the neo-cortex, particularly in combination with the major frontal lobe expansion unique to humans. While the exact function of this expanded cerebellum remains to be established, the sheer size and magnitude of the corticopontocerebellar connections give a clue to its likely involvement in cognitive processes (Leiner et al 1986, 1989). It has been implicated in language processing (Leiner et al, 1991) and there is anatomical evidence supporting a role for the cerebellum in cognition (Middleton and Strick 1994; Schmahmann and Pandya, 1995). Many functional neuroimaging studies demonstrate cerebellar involvement in cognitive processes. In cognitive activation studies that include the

cerebellum, it is common to find increased activity in the cerebellum. One of the pioneer studies on the role of cerebellum in cognition indicated involvement of certain cerebellar regions in processing of sensory information rather than fine motor control (Gao et al, 1996). The cerebellum receives cortical afferents via the pontine relay nuclei. These afferents have recently been discovered to come from more widespread areas of the cortex than was originally thought, and seem to be reciprocal (Schmahmann and Pandya, 1997). Thus, a network exists to support involvement in cognition. Cerebellar lesions have been linked to a cognitive affective syndrome (Schmahmann and Sherman, 1998). This report of twenty patients with disease confined to the cerebellum found striking cognitive and behavioral deficits including difficulties with verbal fluency, working memory, visuospatial organization, personality changes and blunting of affect, in addition to other changes. Cerebellar changes have also been found before in dementia. A recent PET study found decreased cerebellar metabolism in patients with severe Alzheimer's, however they also found significant declines in glucose metabolism throughout the cerebrum (Ishii et al, 1997). The magnitude of the metabolic changes seen was least in the cerebellum, and greatest in the parietal cortex. The cerebellar changes found were only significant in patients with severe Alzheimer's. Another recent examination of patients with olivopontocerebellar atrophy found that this group had deficits in tasks requiring intact frontal and parietal cortices. They postulated that the cerebellum was involved in modulation of these cortical areas, and thus the atrophy had resulted in the cognitive changes seen (Arroyo-Anllo and Botez-Marquard, 1998). A case report of a patient with a cyclic cognitive-affective syndrome examined cerebral perfusion using SPECT (Patterson, 2001). This patient with atypical symptoms of dementia shows increased flow in the cerebellum, which may represent increased activity of the Purkinje cell's inhibitory output, or increased activity of cells upstream to the Purkinje neurons. The increase may have been compensatory, secondary to deficits in other interconnected areas such as the posterior parietal lobe.

In the exemplar study, relative increases in metabolism are reported in the pons, cerebellum, and motor area of patients with MCI. There are previously reported findings of increased cerebellar metabolism in patients with AD (Patterson et al, 2002), and numerous reports of the use of the cerebellum as a reference or control region for normalizing semi-quantitative PET or SPECT data. Indeed, there has been debate on this topic, and at least one study validated the use of the cerebellum as a reference

region in AD (Pickut et al, 1999). Others found either no change (Pickut et al 1999, Soonawala et al, 2002) or decreased cerebellar metabolism in AD (Ishii et al, 1997). One further study found that pontine metabolism was most preserved in patients with AD compared to controls (Minoshima et al, 1995b). The use of a ratio of cerebellar to brain activity is not a novel methodology, in fact it is a standard means of “normalizing” semi-quantitative data (see above section on ROIs). The semi-quantitative results of the instant invention indicate that it is possible that increases found in the pons, cerebellum, and motor strip are the result of global declines in the MCI population, sparing these regions. It is also possible that the metabolic decline found in some regions (posterior cingulate, parietal, etc) results in compensatory activity in other nodes in a network of brain regions. There have been reports in the literature documenting the involvement of the cerebellum in cognition (see Rapoport et al, 2000 for review), so it is not necessarily safe to presume that the cerebellum is uninvolved in disorders involving cognition. The results obtained with the method of the instant invention may not simply be intensity normalization due to global changes, but compensatory increased activity. Whether actual metabolism in these regions is increased or normal, they still serve as optimal regions for the calculation of the CDI.

ROI data based on SPM results has been used to examine functional connectivity between the cerebellum and other regions in a study of acute psychosis and response to antipsychotic medication. Functional connectivity analysis simply looks at the correlation coefficient between two regions (e.g., the cerebellum and the left dorsolateral prefrontal cortex) in a population and compares that value to one obtained in another population. This data has not been published as it contains some admittedly serious confounds. However, the underlying method of using SPM maxima to define ROIs, and then using the ROI data to look at the relationship between two or more brain regions is still valid. The concept of using a region of activity as a locus for an ROI is extant in the literature, and has been used previously (Buchel et al, 1997). This study used the locations of regional maxima from an SPM analysis as seed points for ROIs, and this data was then entered into a Structural Equation Model (SEM) analysis. The SEM and similar methodologies are more advanced than functional connectivity-type correlation analyses, and are called “effective connectivity” analyses. SEM involves the use of a set of mean intensity values derived from ROIs typically taken from specific regions that can be defined by SPM maxima. The underlying concept is based upon

defined anatomical connections, and thus interprets a relationship between two regions as composed of either a direct connection, an indirect connection, or (more commonly) a combination of the two. This is important as the addition of greater than three “nodes” in this network increases the complexity of the calculation by a least an order of magnitude, and this complexity increases in geometric fashion for each node added. One similarity to the method of the instant invention is that weights are assigned to a given “path” between two nodes and used to calculate a path coefficient that represents the strength or activity of the connection between the nodes. This type of analysis is useful to examine the relationship between several regions and how it may change with different cognitive activities. The CDI samples mean ROI intensity data from multiple regions and those values are entered into a formula to calculate CDI. While one component of the formula involves weights, this is not to examine the relationship between regions. While there may be a relationship between certain regions sampled for the CDI, this is not implied, intrinsic, or necessary to the CDI.

As discussed before, three previous reports indicate that it is possible to detect brain metabolic changes using PET across groups of pre-morbid patients, who have not yet developed MCI, before subjective symptoms or neuropsychological impairment occurs (De Leon et al, 2001; Reiman et al, 2001; Small et al, 2000). All three use group analysis to detect changes in groups of subjects. The SPM results used in the instant invention are consistent with these previous reports. One study examined brain scans in post-hoc measures, separating them based upon whether they develop AD later in life. The other two were also longitudinal studies. None of the three present a methodology that enables evaluation and production of a measure usable in a single patient. De Leon and others followed 48 healthy elderly individuals, and scanned them at baseline and again after 3 years. Some of the subjects showed evidence of cognitive decline. By grouping these patients post-hoc, and looking at their first scans as a function of whether they developed cognitive decline at 3 years, they found decreased metabolism in the entorhinal cortex, as well as increased frequency of ApoE4+ genotype. Reiman followed normal subjects who either had or didn't have the ApoE4 phenotype, and scanned twice with a two-year interval. They found that subjects who were ApoE4+ had decreased metabolism in regions of the temporal lobe, posterior cingulate, prefrontal cortex, basal forebrain, parahippocampus, and thalamus, in regions similar to those found in the present study. The study by Small and others was similar. They followed

61 subjects, 54 of whom were aware of mild memory loss, but who were “normal” as determined by cognitive tests. In this population, ApoE4+ genotype was associated with initial decreased metabolism in the posterior cingulate, inferior parietal and lateral temporal areas. These metabolic changes predicted cognitive decline. These studies
5 show that PET scanning using FDG is the most sensitive measure known for the detection of this disorder.

Previous reports have attempted to discriminate patients with early cognitive changes or AD from normal subjects using various methods of objective analysis. A study using a diagnostic index based on parietal lobe Z-scores detected 97% of AD
10 patients (Minoshima et al, 1995a). This same group extended this technique to patients with isolated memory impairment, but were able to detect only 50% (Berent et al 1999). Another report used multiple regression and discriminant analysis to identify 87% of patients with mild to moderate AD and controls (Azari et al, 1993). Another study used logistic regression to identify 95% of AD patients using a combined regressor of FDG
15 metabolic data (from an arbitrarily defined ROI in the left temporoparietal area), along with performance on a “block design” cognitive test (Arnaiz et al 2001). A SPECT study using singular value decomposition and discriminant function analyses was able to detect about 60% of patients with early AD/MCI (Johnson et al, 1998).

The findings from all of these studies can be distinguished from the method of
20 the instant invention by the variability in mathematical approaches, sensitivity, and the use of SPM-derived regional maxima as loci for the VOIs. This method eliminates the confounding condition of arbitrarily defining the lesion site, as well as bypasses the confounding requisite Bonferroni correction for multiple comparisons in SPM. Basing the CDI on multiple VOIs allows for variance across the presentation, while methods
25 that examine only one region (e.g., the parietal lobe) do not. The method of the instant invention also examines 13 major nodes that are believed to be most affected by the processes of AD, based on the a priori knowledge gleaned from the SPM analysis.

There have been two previous studies that used a neural-network method (Kippenham, et al, 1992, 1994) to classify patients with AD from controls. These two
30 reports used neural-network models based on 67 ROIs drawn in all the major regions of the brain. The 1992 study reported that, for patients with possible AD (MMSE of 19 ± 8), the area under the relative operating curve (ROC) was 0.81, similar to that for the clinical evaluation. This was somewhat higher for probable AD (MMSE 15 ± 7) with a

ROC area of 0.85, and improved even more in the 1994 study by using a scanner with higher resolution (ROC area 0.95). One important difference between the Kippenham studies and the present invention is that the present invention uses a priori knowledge of where the pathological regions were (determined with SPM) to sample VOI data. In the instant invention, the SPM methodology is utilized in a unique way, to determine the exact location of regionally significant change for a 3D spherical VOI, bypassing the major drawback of ROI analysis, which is that the ROI is typically drawn arbitrarily. Even when drawn based upon anatomically defined areas, there still is no assurance that an area so defined will respond homogeneously and thus provide a homogeneous response.

PET is a technology that can make use of a variety of radioligands, and is not limited to FDG. AD has been studied with radioligands that bind to cholinergic receptors (e.g., Shinotoh et al, 2003) as well as to neurofibrillary plaques (Shoghi-Jadid et al, 2002). These techniques are quite different than the results presented here as they make use of completely different radioligands and do not examine brain metabolism.

The CDI as described is a marker that can be used to predict AD. As stated above, the marker/method described in United States Patents 5,873,823 and 5,632,276 may be applied to the detection and diagnoses of multiple disease states, including both PD and AD. To apply the teachings of the instant invention to identify and predict the onset of more severe symptoms in other conditions such as PD, the methodology of the instant invention is changed to use different VOIs. This is due to the fact that SPM analysis provides loci specific for MCI, and thus the loci sampled as used in the CDI and in the CDInn are specific for MCI and AD. For diagnoses of PD, an SPM analysis of patients with PD compared to control is first completed in the same manner as described herein. With this requirement established, the following examples illustrate extensions of both the CDI and CDInn to additional clinical presentations.

Parkinson's disease (PD)

PD is a disorder of the brain which affects the dopaminergic neurons of the brainstem first and foremost. By the time that patients first notice a movement disorder or feel the first clinically noticeable signs and symptoms, 50% of the dopamine neurons have been destroyed. Previous reports have shown that there are metabolic changes present in the cerebral cortex as well as subcortical structures in early PD. One specific report by Eidelberg, as reported above, and others used FDG-PET and Scaled Subprofile

Modeling to make predictions about disease states in PD. The description of the methodology in both the Eidelberg patent and the manuscript lacks clarity.

The methodology of the instant patent is applied to gather data in the same fashion with early PD as was done with patients with MCI. Baseline FDG PET scans can be obtained for a group of patients presenting with these symptoms or complaints, and compared to a group of age matched controls using SPM. The SPM statistical data can be used to determine the location (specific coordinates) of regions of significant change. These regions can be sampled with a 5 mm radius VOI using MARSBAR™. Estimates of the frequency of abnormality of each region can be calculated across the patient sample, and used to generate weights for each region. The mean of the weighted VOIs from regions where significant increased metabolism is found can be divided by that from regions with decreased metabolism. The grand mean of this ratio in the control subjects can be adjusted to 1, and the resulting adjustment factor used to normalize all ratios to this standard. This value can be called the "Parkinson's Disease Index," or PDI.

Closed-head Injury (CHI)

CHI is an altogether too common affliction. Patients who have suffered from concussive illnesses often have very little if any objective evidence on an MRI or CT scan to indicate that a traumatic injury has occurred. However, neuropsychological tests and behavioral measures often do find sometimes subtle changes. The purpose of using a PET index in CHI is to provide a definitive and objective measure that can guide treatment and prognosis.

The CDI methodology is further adapted for use in the objective analysis of functional brain data (FDG-PET scans) from patients with CHI. This would involve the use of VOIs from regions that vary on a per-patient basis, as potential metabolic lesions would vary from patient to patient, depending on the location of the traumatic insult and the degree of coup/contre-coup type injury. Baseline FDG PET scans can be obtained for a given patient, and each individual patient can be compared to a group of controls using SPM. The SPM statistical data can be used to determine the location (specific coordinates) of regions of significant change. These regions can be sampled with a 5 mm radius VOI using MARSBAR™. The mean of the VOIs from regions where significant increased metabolism was found can be divided by that from regions with decreased metabolism. The grand mean of this ratio in the control subjects can be

adjusted to 1, and the resulting adjustment factor used to normalize all ratios to this standard.

Substance Abuse

Abuse of dangerous and illicit substances is often found to be associated with pathophysiology in the orbitofrontal cortex and associated limbic and paralimbic regions. While the specific regions may vary with the substance being abused, the rationale remains the same. The purpose of using a PET index in patients who have abused drugs is potentially manifold: to investigate the risk of addiction in certain populations, to study the effect that acute substance abuse or dependency has on cerebral metabolism, and to evaluate populations for lesions who are abstinent but who have abused or were dependent in the past.

Baseline FDG PET scans would be obtained for a group of patients with a history of drug use, and compared to a group of age matched controls using SPM. The SPM statistical data can be used to determine the location (specific coordinates) of regions of significant change. These regions can be sampled with a 5 mm radius VOI using MARSBAR™. Estimates of the frequency of abnormality of each region can be calculated across the patient sample, and used to generate weights for each region. The mean of the weighted VOIs from regions where significant increased metabolism is found can be divided by that from regions with decreased metabolism. The grand mean of this ratio in the control subjects can be adjusted to 1, and the resulting adjustment factor used to normalize all ratios to this standard.

Lewy Body Dementia, Pick's Dementia, Huntington's Disease

Three other less common dementing diseases are Lewy Body Dementia, Pick's Dementia, and Huntington's Disease. These diseases have characteristic metabolic lesion patterns on the PET scan, and thus it is quite feasible to propose Cognitive Decline Indices that are specific for the exact dementia type. The purpose of using a specific PET index for these types of dementia would be several fold: to detect the dementing process as early as possible, to discriminate which type of dementing process it is, and to facilitate the early treatment of these disease processes.

Baseline FDG PET scans can be obtained for a group of patients presenting with these symptoms or complaints, and compared to a group of age matched controls using SPM. The SPM statistical data can be used to determine the location (specific coordinates) of regions of significant change. These regions can be sampled with a 5

mm radius VOI using MARSBAR™. Estimates of the frequency of abnormality of each region can be calculated across the patient sample, and used to generate weights for each region. The mean of the weighted VOIs from regions where significant increased metabolism was found can be divided by that from regions with decreased metabolism.

- 5 The grand mean of this ratio in the control subjects can be adjusted to 1, and the resulting adjustment factor used to normalize all ratios to this standard.

In developing the CDInn for any of these conditions, a patient's CDInn is compared to established normal ranges of values. The presence of normality or abnormality is determined from the CDI value 500 or from the CDInn. If the CDInn
10 reading is negative, the patient is advised and educated about the clinical course of potential illnesses and told the signs to watch for. The potential benefits of preventative measures including anti-oxidants, mental exercises, beneficial diet and adequate rest are discussed 510. If the CDInn reading is positive, the patient is educated about the meaning of the positive reading, and informed about the projected clinical course of the
15 illness. The benefits of medication, and the potential benefit of ameliorative measures such as anti-oxidants, mental exercises, beneficial diet and adequate rest are discussed. 520. In either case, results are given to the referring physician and the patient is scheduled for re-evaluation 530. Patient data is stored in the comprehensive patient database 540.

20 The above descriptions of the exemplary embodiments of methods for the determination of clinical conditions and for the quantitative description of metabolically correlated brain function are for illustrative purposes. Those skilled in the art that have the benefit of this disclosure will recognize that certain changes can be made to the component parts of the method of the present invention without changing the manner in
25 which those parts function to achieve their intended result. The instant invention may also be practiced in the absence of any element not specifically disclosed. All such changes, and others which will be clear to those skilled in the art from this description of the preferred embodiments of the invention, are intended to fall within the scope of the following, non-limiting claims.

References

- Arnaiz E, Jelic V, Almkvist O, Wahlund L, Winblad B, Valind S, and Nordberg A (2001). Impaired cerebral glucose metabolism and cognitive functioning predict deterioration in mild cognitive impairment. *Neuroreport*, 12(4):851-855.
- 5 Arroyo-Anillo EM and Botez-Marquard TB: Neurobehavioral Dimensions of Olivopontocerebellar Atrophy. *J Clin Exp Neuropsychol*. 1998; 20:52-59.
- Azari NP, Pettigrew KD, Schapiro MB, Haxby JV, Grady CL, Pietrini P, Salerno JA, Heston LL, Rapoport SI, Horwitz B. Early detection of Alzheimer's disease: a statistical approach using positron emission tomographic data. *J Cereb*
 10 *Blood Flow Metab* 1993 May;13(3):438-47
- Berent S, Giordani B, Foster N, Minoshima S, Lajiness-O'Neill R, Koeppe R, Kuhl DE. Neuropsychological function and cerebral glucose utilization in isolated memory impairment and Alzheimer's disease. *J Psychiatr Res* 1999 Jan-Feb;33(1):7-16
- 15 Boellaard R, van Lingen A, and Lammertsma A (2001). Experimental and Clinical Evaluation of Iterative Reconstruction (OSEM) in Dynamic PET: Quantitative Characteristics and Effects on Kinetic Modeling. *J Nucl Med*, 42:808-817.
- Brett M, Anton JL, Valabregue R, Poline JB (2002). Region of interest
 20 analysis using an SPM toolbox [abstract] Presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2-6, 2002, Sendai, Japan. *NeuroImage*, Vol 16, No 2.
- Brookmeyer R, Gray S, Kawas C. (1998). Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. *Am J*
 25 *Public Health*. 88(9):1337-42.
- Buchel C, & Friston K J, (1997). Modulation of connectivity in visual pathways by attention: cortical interactions evaluated with structural equation modelling and fMRI. *Cerebral Cortex*, 7, 768-778.
- Chen P, Ratcliff G, Belle SH, Cauley JA, DeKosky ST, Ganguli M, (2001).
 30 Patterns of Cognitive Decline in Presymptomatic Alzheimer Disease: A Prospective Community Study. *Arch Gen Psych*, 58:853-858.

Collie A, Maruff P. An analysis of systems of classifying mild cognitive impairment in older people. *Aust N Z J Psychiatry* 2002 Feb;36(1):133-40

De Leon MJ, Convit A, Wolf OT, Tarshish CY, DeSanti S, Rusinek H, Tsui W, Kandil E, Scherer AJ, Roche A, Imossi A, Thorn E, Bobinski M, Caraos C, Lesbre P, Schlyer D, Poirier J, Reisberg B, Fowler J. (2001). Prediction of cognitive decline in normal elderly subjects with 2-[(18)F]fluoro-2-deoxy-D-glucose/positron-emission tomography (FDG/PET). *Proc Natl Acad Sci U S A*. 98(19):10966-71.

De Santi S, de Leon MJ, Rusinek H, Convit A, Tarshish CY, Roche A, Tsui WH, Kandil E, Boppana M, Daisley K, Wang GJ, Schlyer D, Fowler J. (2001). Hippocampal formation glucose metabolism and volume losses in MCI and AD. *Neurobiol Aging*, 22(4):529-539.

Eidelberg D, Moeller JR, Ishikawa T, Dhawan V, Spetsieris P, Chaly T, Belakhlef A, Mandel F, Przedborski S, Fahn S. (1995a). Early differential diagnosis of Parkinson's disease with 18F-fluorodeoxyglucose and positron emission tomography. *Neurology*, 45(11):1995-2004.

Eidelberg D, Moeller JR, Ishikawa T, Dhawan V, Spetsieris P, Chaly T, Robeson W, Dahl JR, Margouleff D. (1995b). Assessment of disease severity in parkinsonism with fluorine-18-fluorodeoxyglucose and PET. *J Nucl Med*. 36(3):378-83.

Evans DA, Funkenstein HH, Albert MS, Scherr PA, Cook NR, Chown MJ, Hebert LE, Hennekens CH, Taylor JO. (1989). Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. *JAMA*, 262(18):2551-6.

Folstein MF, Folstein, SE and McHugh PR (1975) Mini-Mental State: A practical method for grading the state of patients for the clinician, *Journal of Psychiatric Research*, 12: 189-198.

Fox PT, Raichle ME, Mintun MA, Dence C (1988). Nonoxidative glucose consumption during focal physiologic neural activity. *Science* 241:462-464.

Friston KJ, Ashburner J, Frith CD, Poline JB, Heather JD, and Frackowiak RSJ (1995a). Spatial registration and normalization of images. *Human Brain Mapping*, 2:165-189.

Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith CD, and Frackowiak RSJ (1995b). Statistical parametric maps in functional imaging: A general linear approach. *Human Brain Mapping*, 2:189-210.

5 Gao JH, Parsons LM, Bower JM, Xiong J, Li J, Fox PT (1996). Cerebellum implicated in sensory acquisition and discrimination rather than motor control. *Science*, 272(5261):545-7.

Ibanez V, Pietrini P, Alexander GE, Furey ML, Teichberg D, Rajapakse JC, Rapoport SI, Schapiro MB, Horwitz B. Regional glucose metabolic abnormalities are not the result of atrophy in Alzheimer's disease. *Neurology* 1998 Jun;50(6):1585-93

10 Ishii K, Sasaki M, Kitagaki H, Yamaji S, Sakamoto S, Matsuda K, Mori E. Reduction of cerebellar glucose metabolism in advanced Alzheimer's disease. *J Nucl Med*. 1997 Jun;38(6):925-8.

Johnson KA, Jones KJ, Becker JA, Satlin A, Holman BL, Albert MS (1998). Preclinical prediction of Alzheimer's disease using SPECT. *Neurology*, 50:1563-1571.

Katzman R, Kang D, Thomas R. (1998). Interaction of apolipoprotein E epsilon 4 with other genetic and non-genetic risk factors in late onset Alzheimer disease: problems facing the investigator. *Neurochem Res*. 1998 Mar;23(3):369-76.

20 Kippenhan JS, Barker WW, Nagel J, Grady C, Duara R. Neural-network classification of normal and Alzheimer's disease subjects using high-resolution and low-resolution PET cameras. *J Nucl Med*. 1994 Jan;35(1):7-15.

Kippenhan JS, Barker WW, Pascal S, Nagel J, Duara R. Evaluation of a neural-network classifier for PET scans of normal and Alzheimer's disease subjects. *J Nucl Med*. 1992 Aug;33(8):1459-67.

25 Kirby M, Denihan A, Bruce I, Coakley D, Lawlor BA. The clock drawing test in primary care: sensitivity in dementia detection and specificity against normal and depressed elderly. *Int J Geriatr Psychiatry*. 2001 Oct;16(10):935-40.

Leiner HC, Leiner AL, Dow RS (1986): Does the cerebellum contribute to mental skills? *Behav Neurosci*, 100(4):443-54.

30 Leiner HC, Leiner AL, Dow RS (1989): Reappraising the cerebellum: what does the hindbrain contribute to the forebrain? *Behav Neurosci*, 103(5):998-1008.

Leiner HC, Leiner AL, Dow RS (1991): The human cerebro-cerebellar system: its computing, cognitive, and language skills. *Behav Brain Res*, 44(2):113-28.

Meltzer CC, Zubietta JK, Brandt J, Tune LE, Mayberg HS, Frost JJ. Regional hypometabolism in Alzheimer's disease as measured by positron emission tomography after correction for effects of partial volume averaging. *Neurology*. 1996 Aug;47(2):454-61.

Middleton FA and Strick PL (1994): Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. *Science*, 266(5184):458-461.

Minoshima S, Frey KA, Koeppe RA, Foster NL, Kuhl DE. A diagnostic approach in Alzheimer's disease using three-dimensional stereotactic surface projections of fluorine-18-FDG PET. *J Nucl Med*. 1995 Jul;36(7):1238-48.

Minoshima S, Frey KA, Foster NL, Kuhl DE. Preserved pontine glucose metabolism in Alzheimer disease: a reference region for functional brain image (PET) analysis. *J Comput Assist Tomogr*. 1995b Jul-Aug;19(4):541

Nadeau SE, Crosson B (1995). A guide to the functional imaging of cognitive processes. *Neuropsychiatry, Neuropsychology and Behavioral Neurology* 8:143-162.

Parsons LM and Fox PT: Sensory and cognitive functions. *Int Rev Neurobiol* 1997; 41:255-271.

Patterson JC (2001): Cerebellar perfusion abnormalities correlated with change in cognitive and affective state in a 78-year-old man. *Am J Geriatr Psychiatry*. 2001 Summer;9(3):309-14.

Patterson JC, Tainter KH, Lilien DL, and Glabus MF. (2002). Increased Cerebellar Metabolism in Alzheimer's disease. (abstract). Presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2-6, 2002, Sendai, Japan. Available on CD-Rom in *NeuroImage*, Vol. 16, No. 2.

Petersen RC, Stevens JC, Ganguli M, Tangalos EG, Cummings JL, DeKosky ST. (2001). Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*. 56(9):1133-1142.

Pickut BA, Dierckx RA, Dobbeleir A, Audenaert K, Van Laere K, Vervaet A, De Deyn PP. Validation of the cerebellum as a reference region for SPECT

quantification in patients suffering from dementia of the Alzheimer type. *Psychiatry Res.* 1999 Apr 26;90(2):103-12.

Rapoport M, van Reekum R, Mayberg H (2000). The Role of the Cerebellum in Cognition and Behavior: A Selective Review. *J Neuropsych Clin Neurosci* 12:193-198.

Reiman EM, Caselli RJ, Chen K, Alexander GE, Bandy D, Frost J. (2001). Declining brain activity in cognitively normal apolipoprotein E epsilon 4 heterozygotes: A foundation for using positron emission tomography to efficiently test treatments to prevent Alzheimer's disease. *Proc Natl Acad Sci U S A.* 98(6):3334-9.

Russell JM, Early TS, Patterson JC, Martin JL, Villanueva-Meyer J, McGee MD, (1997). Temporal lobe perfusion asymmetries in schizophrenia. *J Nucl Med*, 38(4):607-12.

Schmahmann JD and Pandya DN (1995). Prefrontal cortex projections to the basilar pons in rhesus monkey: implications for the cerebellar contribution to higher function. *Neurosci Lett*, 199(3):175-8.

Schmahmann JD and Pandya DN: The cerebrocerebellar system. *Int Rev Neurobiol.* 1997; 41:31-60.

Schmahmann JD and Sherman JC: The cerebellar cognitive affective syndrome. *Brain* 1998; 121:561-579.

Schwartz WJ, Smith CB, Davidsen L, Savaki H, Sokoloff L, Mata M, Fink DJ, Gainer H (1979). Metabolic mapping of functional activity in the hypothalamo-neurohypophysial system of the rat. *Science* 205:723-725.

Shinotoh H, Fukushi K, Nagatsuka S, Tanaka N, Aotsuka A, Ota T, Namba H, Tanada S, Irie T (2003). The amygdala and Alzheimer's disease: positron emission tomographic study of the cholinergic system. *Ann N Y Acad Sci*, 985:411-419.

Shoghi-Jadid K, Small GW, Agdeppa ED, Kepe V, Ercoli LM, Siddarth P, Read S, Satyamurthy N, Petric A, Huang SC, Barrio JR (2002). Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease. *Am J Geriatr Psychiatry* 10(1):24-35.

Shulman K, Shedletsky R, Silver I. The challenge of time: clock-drawing and cognitive function in the elderly. *Int J Geriatr Psychiatry* 1986;1:135-40.

Silverman DH, Gambhir SS, Huang HW, Schwimmer J, Kim S, Small GW, Chodosh J, Czernin J, Phelps ME. Evaluating early dementia with and without
5 assessment of regional cerebral metabolism by PET: a comparison of predicted costs and benefits. *J Nucl Med*. 2002 Feb;43(2):253-66.

Small GW, Ercoli LM, Silverman DH, Huang SC, Komo S, Bookheimer SY, Lavretsky H, Miller K, Siddarth P, Rasgon NL, Mazziotta JC, Saxena S, Wu HM, Mega MS, Cummings JL, Saunders AM, Pericak-Vance MA, Roses AD, Barrio JR,
10 Phelps ME. (2000). Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A*. 97(11):6037-42.

Soonawala D, Amin T, Ebmeier KP, Steele JD, Dougall NJ, Best J, Migneco O, Nobili F, Scheidhauer K. Statistical parametric mapping of (99m)Tc-HMPAO-SPECT images for the diagnosis of Alzheimer's disease: normalizing to cerebellar
15 tracer uptake. *Neuroimage*. 2002 Nov;17(3):1193-202.

Talairach J and Tournoux P, (1988). *Co-Planar Stereotactic Atlas Of The Human Brain*. Stuttgart: Georg Thieme Verlag.

Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, and Evans AC (1996). A unified statistical approach for determining significant voxels in images of
20 cerebral activation. *Human Brain Mapping*. 4:58-73.